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A NEWLY INVENTED CHEMICAL DISINFECTANT CAN ERADICATE COLONIC BACTERIAL CONTAMINATION OF THE SKIN

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ABSTRACT

To assess the antibacterial effect of a new chemical solution made by a mixing Sidr ziziphus spina christi infusion with hydrogen peroxide (H2O2).

The antibacterial effect of the mixture of sidr infusion with H2O2 were evaluated through surface disinfectant testing microscopic slides were used as carriers. Twenty sterile slides were contaminated through immersion into diluted faeces. Having been washed and rinsed by sterile tap water, each pair of slides was soaked for 2 minutes into one of the 20 solutions; sidr (2.5, 5.0, 6.25, 7.5, 8.75, 10.0) g% and H2O2 (1.5, 3)%, mixture of H2O2 1.5% and 3% with all sidr infusion concentrations separately. The slides were divided equally to be cultivated in nutrient agar or Bacteroides Bile Escolin agars (BBE). The media were incubated for 18-48 hours in aerobic or anaerobic conditions as required. Room temperature was 35-45 $^{\circ}$C.

The study then involved 5 adults as volunteers, after contaminating their hands through immersion for 15 minutes into a basin of liquid stool. Having been air dried, the hands were washed by neutral soaps for 1 minute, then bacteriological swabs were taken which were cultivated for 18-48 hours, under aerobic or anaerobic conditions as required. Guided by the slides testing results, the hands were then immersed into 3 selected solutions (sidr 6.25 g%, H2O2 1.5%, and mixture of both) using one solution only in each step for 2 minutes of immersion. Shifting to the other solutions was only done after an interval of 30 minutes (for cleaning, rinsing and drying the hands). Each time swabbing then dipping finger tips into nutrient agars were performed to be followed by cultivation both aerobically or anaerobically.

Results of surface disinfectant testing (microscopic slides) and results of cultivated agars indicated that significant variable growth of bacterial colonies were visible in all cultures except the conditions when the mixture of both sidr infusion 6.25g% and H2O2 1.5% was used We concluded that the newly invented chemical disinfectant can eradicate all colonic bacterial contamination from the skin and inanimate surfaces to be listed among hospital disinfectants.

KEYWORDS: Colonic Bacteria, Nosocomial Infection, Extract, Disinfectant

INTRODUCTION

In the USA, as well as in several other high income countries, device-associated healthcare-associated infection surveillance in the intensive care unit (ICU) plays a substantial role in hospital infection control and quality assurance[1]. One third of nosocomial infections are preventable. The CDC estimates 2 million people in USA are infected annually by hospital-acquired infections, resulting in 20,000 deaths[2]. The most commn nosocomial infections are of the urinary tract, surgical site and various pneumonias[3] are known to survive on inanimate 'touch' surfaces for extended periods of time[4].

The human colon has the largest population of bacteria in the body (in excess of 10^{11} organisms per gram of wet weight), and the majority of these organisms are anaerobes[5]. Intestinal micro biota includes about 800 different bacterial species with over 7000 strains[6]. The majority of these species, a large number of which is uncultivable with available media, has been identified using molecular methods based on sequencing bacterial ribosomal RNA(16S rRNA) genes and is represented by obligate anaerobes[7,8]. The most common Anaerobic genera are *Bacteroides*, *Eubacterium*, *Fusobacterium*, *Clostredium* and *Lactobacillus*, whereas aerobes are Gram –negative enteric bacteria (such as *Escherichia coli* and *Salmonella spp*.) and Gram –positive cocci such as *Enterococcus*, *Staphylococcus* and *Streptococcus*); moreover, anaerobic infections fungal species are also present, such as Candida albicans[9]. Non- sporing anaerobic, rod shaped organisms overwhelmingly predominate in the human faecal flora, accounting for more than 99% of the total organisms[10].

Unlike the general coliform group, *E. coli* are almost exclusively of fecal origin and their presence is thus an effective confirmation of fecal contamination[11].

In the most serious infections caused by anaerobic bacteria, a consensus group of infectious disease physicians have concluded that patient clinical response does, in fact, correlate with *in-vitro* antimicrobial susceptibility testing results[12].

The drug-resistant Gram-negative bacteria, for the most part, threaten only hospitalized patients whose immune systems are weak. They can survive for a long time on surfaces in the hospital and enter the body through wounds, catheters, and ventilators[13]. Hydrogen peroxide (HP) is an active agent that affects a wide range of organisms such as bacteria, yeast, fungi, viruses, and spores. The antibacterial effect of HP involves hydroxyl radicals. The hydroxyl radical, being a potent oxidant, can react easily with macromolecules such as membrane lipids and DNA thus resulting in bacterial death[14]. It is evident that *Z. spina-christi* has pharmacological functions, including antihyperglycemic, antibacterial, antifungal, antioxidant and antinociceptive activities, among others[15].

MATERIAL AND METHODS

A branch of sidr tree was introduced to taxonomy department in college of Science/Babylon University. The bacteriological tests were done in the Biology Department Laboratory/coll. of science/Babylon University and in Public Health Laboratory/Babylon Health Directorate. The pH of the sidr infusion was determined using digital pH meter.

Physical Stability Test: One liter of both sidr infusion and H2O2 was stored for three months in the refrigerator. After three months, their stability was assessed regarding appearance and pH in addition to antibacterial activity.

Preparation of Sidr infusion

The fresh sidr leaves after being cleaned and washed in tape water (2.5, 5.0, 6.25, 7.5, 8.75 and 10.0) gs. were collected in separate containers. 100 ml of distilled water was added to each container and all the samples were heated until boiling. We left the mixture for 12 hrs, rubbing the leaves with the gloved hands, the infusion was collected to be kept in the refrigerator for use.

Preparation of Hydrogen Peroxide

Hydrogen peroxide 6% liquid solution made by Baghdad Factory, diluted by distilled water to 3%and 1.5%. A sample from each concentration was put in separate beakers.

In Test-Use

To exclude bacterial contamination of the test solutions; sidr infusions, H2O2.1ml of the test solution was put into a tube containing 9 ml of nutrient broth using a pipette 50 drops /ml.10 drops were dispersed separately on two nutrient agar plates. One plate was incubated at 37 $^{\circ}_{C}$ while the other at 25 $^{\circ}_{C}$ for 72 hrs. The incubated plates were inspected for evidence of growth, 1 drop was turbid in each plate which was compatible with absence of bacterial contamination of the stalk solutions[16]

Surface Disinfectant Testing

The carriers we used to simulate a hard non porous inanimate surface were microscopic slides according to[17]. The carriers were inoculated with the test microbes (colonic flora) by submergence in diluted feces for 15 minutes. After they air dried, 1 pair of the slides were placed into 10 ml of one of the 20 solutions sidr infusion (2.5, 5.0, 6.25, 7.5, 8.75 and 10.0) g %, H2O2 1.5% and 3% -mixture of sidr infusion with H2O2 in all dilutions) Table 1. These slides were cultivated in a nutrient and MacConky agars and swabs were streaked on Bacteroides Bile Escolin agar (BBE), incubated for 18-48 hours in aerobic and anaerobic conditions. The range of the temperature of the solutions was 35-45$^{\circ}_{C}$.

Testing Cell Toxicity

After fruitful results of the surface disinfectant testing and prior to application on human we evaluated cell toxicity. We prepared phosphate buffer saline solution 500mg/ml, 2mlof blood were withdrawn from a volunteer and put into a test tube containing an anticoagulant to which one ml of the phosphate buffer saline solution alone as a negative control and tape water as positive control. 0.8ml of sidr infusion 6.25 g% was put in a sterile test tube to which 0.2 ml of red blood cells. The test tubes were incubated at 37 for 3 hrs during which the solution was examined hourly for evidence of hemolysis. All the microscopic slides showed no hemolysis indicating that no cell toxicity resulted from use of sidr infusion[18].

The study then involved 5 male adults as volunteers. One of them was selected to bring a sample of his stool for having neither gastrointestinal problem nor taking a course of antibacterial drug within last 3 months 20 grams of the stool were diluted by 2 liters of normal saline and stirred to be homogenous solution. After contaminating their hands through immersion for 15 minutes into a basin of the liquid stool they were air dried, then washed by neutral soaps for 1 minute. Having been rinsed, the hands were swabbed and finger tips were dipped into nutrient agars which were cultivated for 18-48 hrs, under aerobic and anaerobic conditions. Guided by the results of the above mentioned slides testing, the hands then immersed into 3 selected solutions (sidr infusion 6.25 g%, H2O2 1.5%, mixture of both solutions. Using one of the 3 solutions only in each step for 2 minutes of immersion. Shifting to the following solution was only done after an interval of 30 minutes (for cleaning, rinsing and drying the hands). Each time hands contamination, cleaning, rinsing followed by swabbing and dipping finger tips into nutrient agars were performed to be followed by cultivation both aerbically and an aerobicall. We tested the susceptibility of *Staphyllococcus aureus* and *E. coli*. and *Pseudomonus aeroginosa* to each of the disinfectants separately; H2O2 1.5%, sidr infusion 6.25g%, mixture of both (H2O2 1.5%, sidr infusion 6.25g% The bacterial isolates were obtained from clinical cases admitted to Hilla Teaching General Hospital. The agar was incubated for 24 hours at 37 $^{\circ}$C. The same steps were applied when the other isolates were tested.

A nutrient agar was streaked with isolates of *Staphyllococcus aureus*, then 3 wells of 6mm diameter were performed. Every well was filled by 0.5 ml from one of the 3 disinfectant solutions.

Testing stability and long term effectiveness of the antibacterial mixture: we disperse sidr infusion 6.25g%, H2O2 1.5%, mixture of sidr infusion with H2O2 in three sterile test tubes separately. The solutions were incubated at 25 for 1 week separately, on the 8th day, the solutions were a swab was streaked on a nutrient agar and cultivated for 24 hours, inspecting the media revealed growth of bacteria. The same procedure was repeated adding 0.5 ml glycerin 10% to each test tube to the stalk solution, no bacterial growth was seen in case of sidr infusion alone while the growth was still in case of the mixture. The results indicated that sidr was able to keep function when it was stored for long time after addition of glycerin while the function was no longer when it was mixed with H2O2.

RESULTS

The results of testing the fecal contaminated slides after immersion into the proposed and known disinfectants indicated that the only solution that inhibited or killed all cultivable colonic bacteria was the mixture made by mixing sidr infusion 6.25 g % with H2O2 1.5% (table 1).

Table 1: The Effects of Various Disinfectants on Faecal Contaminated Slides (Surface Disinfectant Test)

Serial Number	Disinfectant	Bacterial Growth	
		Aerobic Incubation	Anaerobic Incubation
1	H2O2 3%	GROWTH	GROWTH
2	SIDRE INFUSION 2.5g %	GROWTH	GROWTH
3	SIDRE INFUSION 5 g%	GROWTH	GROWTH
4	SIDRE INFUSION 6.25 g %	GROWTH	GROWTH
5	SIDRE INFUSION 7.5 g %	GROWTH	GROWTH
6	SIDRE INFUSION 8.75g %	GROWTH	GROWTH
7	SIDRE INFUSIO 10 g%	GROWTH	GROWTH
8	H2O2 1.5%	GROWTH	GROWTH
9	Mixture Serial No. 8 +2	GROWTH	GROWTH
10	Mixture Serial No. 8 +3	GROWTH	GROWTH
11	Mixture Serial No. 8 +4	NO GROWTH	NO GROWTH
12	Mixture Serial No. 8 +5	GROWTH	GROWTH
13	Mixture Serial No. 8 +6	GROWTH	GROWTH
14	Mixture Serial No. 8 +7	GROWTH	GROWTH
15	Mixture Serial No. 1 +2	GROWTH	GROWTH
16	Mixture Serial No. 1 +3	GROWTH	GROWTH
17	Mixture Serial No. 1 + 4	GROWTH	GROWTH
18	Mixture Serial No. 1 +5	GROWTH	GROWTH
19	Mixture Serial No. 1 +6	GROWTH	GROWTH
20	Mixture Serial No. 1 +7	GROWTH	GROWTH

Three bacterial isolates; *E. coli*, *S. aureous*, and *Pseudomonus aeroginosa* grown from clinical cases admitted in Hilla Teaching Hospital were tested for their susceptibility to the 3 solutions; sidr 6.25 g %, H2O2 1.5%, mixture of sidre with H2O2. We selected these organisms according to EPA (Environment Protection Agency) registration efficacy standards for hospital disinfectants The extracts from *Z. spina-christi* could be useful in the treatment of nosocomial infections, opportunistic infection of the urinary tract (UTI) [15].The incubated bacterial isolates were inspected after 24 hours. The sensitivity was only seen when the mixture of sidr 6.25 g % with H2O2 1.5 % was used, recording wide inhibition zones among all types of grown bacterial colonies as shown in (table 2).

Table 2: Inhibition Zones of Sidr, H2O2, and Mixture (Sidr & H2O2) at the Mentioned Concentrations on Some Bacteria

Bact. Isolate	Sidr & H2o2	H2O2 1.5%	Sidr 6.25%
E. coli	26 mm	R	4mm
S. aureous	28mm	R	R
Pseudomonus aeroginosa	18 mm	R	R

R: Resistance

Stability determination of sidr leaves infusion showed stable homogenous appearance.

The pH of mixture of the sidr infusion with hydrogen peroxide was 5.98.

DISCUSSIONS

Surface disinfectant testing pointed that among the tested solutions proposed or known disinfectants, only the mixture of sidre 6.25 % with H2O2 1.5 % was able to eradicate all colonic bacteria from the fecal contaminated slides.

We had tested the antibacterial effects of the different disinfectants through five volunteers at the same time and environmental conditions instead of repeating the tests on the same person and to have only one source of contamination e.g. diluted stool of the same person, and to avoid results bias if different environmental conditions were used and to shorten the time required for performing the multiple procedures.

Oral administration of *Z. spina-christi* leaf extract, plain and formulated for 28 days, reduced blood glucose level with significant increase in serum insulin and C-peptide levels [15].

In the most serious infections caused by anaerobic bacteria, a consensus group of infectious disease physicians have concluded that patient clinical response does, in fact, correlate with *in-vitro* antimicrobial susceptibility testing results[4], clearly supporting the use of these tests in selected cases.

Micro-organisms are known to survive on inanimate 'touch' surfaces for extended periods of time[4].

Touch surfaces commonly found in hospital rooms, such as bed rails, call buttons, touch plates, chairs, door handles, light switches, grab rails, intravenous poles, dispensers (alcohol gel, paper towel, soap), dressing trolleys, and counter and table tops are known to be contaminated with *Staphylococcus*, MRSA (one of the most virulent strains of antibiotic-resistant bacteria) and vancomycin-resistant *Enterococcus* (VRE)[19]. One-third of nosocomial infections are considered preventable. The CDC estimates 2 million people in the United States are infected annually by hospital-acquired infections, resulting in. The most common nosocomial infections are of the 20,000 deaths, surgical site and urinary tract various pneumonias[17]. The extract stability was confirmed i,e preserving its pH and color and antibacterial activity.

Cell toxicity was excluded that no hemolysis was seen after three hours of contact between the high concentration of the sidr infusion with human red blood cells. The frequency of micronuclei did not increase and the plant was found devoid of clastogenic activity. Teratogenicity data revealed no teratogenic or fetotoxic effects in the study. Oral LD50 values were determined for Zizyphus spina–christi (>6400 mg/kg)[20].

The main toxic effect resulting from exposure to hydrogen peroxide is irritation at the site of contact[21]. Most cases of acute ingestion of hydrogen peroxide result only in mild adverse effects[22].

CONCLUSIONS

The newly invented chemical disinfectant made by mixing sidre infusion 6.25mg% with hydrogen peroxide 1.5% can eradicate all colonic bacterial contamination from the skin and inanimate surfaces such as touch surfaces commonly found in hospital rooms, such as bed rails, call buttons, touch plates, chairs, door handles, light switches in addition to dressing trolleys and table tops which are known to be contaminated with an important source of hospital acquired infections. It may be considered as a standard hospital disinfectant being demonstrated to be effective against 2 nosocomial pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, to meet EPA registration efficacy standards.

REFERENCES

1. Edwards, J. R.; Peterson, K.D.; Mu, Y.; Banerjee, S.; Allen-Bridson, K. and Morrell, G., *et al*. (2009). National Healthcare Safety Network report. Am J Infect Control; 37:783-805.
2. Ricks, D. (2007). "Germ Warfare". *Ms. Magazine*: 43–5.
3. Klevens, R. M.; Edwards, J.R. and Richards, C.L, *et al.* (2007). "Estimating health care-associated infections and deaths in U.S. hospitals, 2002". *Public Health Rep* **122** (2): 160–6.
4. Wilks, S. A.; Michels, H. and Keevil, C.W. (2005). The Survival of *Escherichia Coli* O157 on a Range of Metal Surfaces, International Journal of Food Microbiology, Vol. 105, pp. 445–454.
5. Salyers, A. A. (1984). Bacteroides of the human lower intestinal tract. Annu. Rev. Microbiol.38:293-313.
6. Ley, R.E.; Peterson, D.A. and Gordon, J.I. (2006). Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell; 124:837–48.
7. Gustafsson, B. E. (1982). The physiological importance of the colonic microflora. Scand *J* Gastroenterol Suppl; 77:117–31.
8. Suau, A.; Bonnet, R. and Sutren, M., *et al*.(1999). Direct analysis of genes encoding 16S rRNA from complex communities reveals many Znovel molecular species within the human gut. Appl Environ Microbiol; 65: 4799–807.
9. Noverr, M.C. and Huffnagle, G.B. (2004).Does the microbiota regulate immune responses outside the gut? Trends Microbiol; 12:562–8.
10. Peach, S.; Fernandez, F.; Johnson, K. and Drasar, B. S. (1974). The non-sporing anaerobic bacteria in human facces. *J. med. Microbiol*., 7, 213-221.
11. Rosenblatt, J. E. and Brook, I. (1993). Clinical relevance of susceptibility testing of anaerobic bacteria. Clin Infect Dis, 16 (Suppl 4): S446–S448.
12. Pollack, A. (2010). "Rising Threat of Infections Unfazed by Antibiotics" New York Times, Feb. 27.

13. Block, B.B.(1991). Peroxygen compounds. In: Block SS, editor. *Disinfection, Sterilization, and Preservation*. 4th edition. Philadelphia, Pa, USA: Lea & Febiger; p. 167.
14. International Programme on Chemical Safety (IPCS).(1998). *Hydrogen Peroxide Poisons Information Monograph. PIM 946*. WHO: Geneva.
15. Jinous, A. and Elaheh, H. (2010). Phytochemistry and pharmacologic properties of *Ziziphus spina christi* (L.) Willd. African Journal of Pharmacy and Pharmacology Vol. 6(31), pp. 2332-2339.
16. Randhawa,V. S. Geeta, M. and Sharma, K.B. (2007). Elsevier In Use test for disinfectants in Practical and viva in medical microbiology.
17. AOAC International (2009) AOAC Official Method 955.15 *Testing disinfectants against Staphylococcus aureus: Use-dilution Method*. Gaithersburg, MD, USA.
18. Dhari, J.; Mohammed, N. and Mehdi, P. (2007).Evaluate the effectiveness of the antibacterial activity of alcoholic and aqueous extracts of leaves of Sidr. Basrah Journal of Science (B). Volume 25, 1: 1-16 (the article was published in Aarabic language).
19. Hospital disinfectants must be demonstrated to be effective against 2 nosocomial pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, to meet EPA registration efficacy standards. Previously, testing against *Salmonella enterica* was also necessary for a hospital disinfectant claim, but this requirement was remove in 2012.TM Standardization News, pp. 28-31.
20. Islam, M.W., Radhakrishnan R., Liu X.M., Chen H.B. and Al-Naji M.A. (2001).Safety evaluation of Zizyphus spina-Christi L. and Teucrium stocksianum Boiss, used in traditional Medicine in the Arabian gulf. International Congress and 49th Annual Meeting of the Society for Medicinal Plant Research (Gesellschaft for Arzneipfianzenforschung), September 2-6.
21. Canadian Centre for Occupational Health and Safety (CCOHS). (1998), *Cheminfo: Hydrogen Peroxide solutions 35% and greater. Record number 198.*
22. International Programme on Chemical Safety (IPCS), (1998). Hydrogen Peroxide. Poisons Information Monograph. PIM 946.